International Journal of Applied and Universal Research **E- ISSN No: 2395-0269**
Volume VI, Issue I, February 2019 Available online at: www.ijaur.com

ESTIMATION OF AMINO ACIDS WITH BRCL REAGENT ON MICRO SCALE

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ABSTRACT: Amino acids are the building blocks of which proteins are made up of simple proteins contain only amino acids while conjugated proteins have additional compounds. In principle, the term "amino acids" could be used to refer to any compound containing an amino group and acidic function. Amino acids are of main importance in the metabolism of all organism. Because, they are the precaursors of proteins.
It is interesting to note that different organisms vary considerably in their ability to synthesise amino acids. For instance, man and the albinorat can synthesise 10 of the 20 essential amino acids required as building blocks of proteins.
However, the remaining acids must be obtained from plants or bacteria. About 20 amino acids are commonly present in proteins. The synthesis of a number of these protein amino acids from the intermediates of glucose metabolism is indicated by in figure and table.

KEYWORDS-: cysteine, methionine and Cystine, BrCl reagent, Iodometrically.

INTRODUCTION:-
Berko and Zyka estimated cysteine chloride by titrating it with periodate in hydrochloric acid. Suchomelove and Zyka determined cysteine thiourea and thiosemicarbazide by oxidation with Pb (IV) acetate. Microdetermination of sulphur containing aminoacids and proteins by iodine-titration has been suggested by different investigators like Horoner Okuda and Talmud Karl Heinzgensch developed an improved iodometric method for the determination of methionine. Thibert and Coworkers estimated the trace of sulphur containing aminoacids with N-bromosuccinimide using bordeaux red as indicator. Christoph determined cysteine as cystine acid.

Ryadohikov estimated sulphur containing aminoacids by oxidation with performic acid in which methionine is oxidised to sulphone and cysteine to cysteic acid. Beg and Shukla determined sulphur containing aminoacids with ammonium hexa nitratocerate (IV) as the oxidant. Ravi Prakash used bromine mono-chloride for the determination. Many chemists determined thioamino acids with using different reagent in different conditions.

PRESENT WORK : In the present chapter, a quick and convenient method has been adopted for the microdetermination of a few thioamine acid, viz., Cysteine, methionine and Cystine with Brcl reagent. The method is of general applicability and percentage error does not exceed ±1.0. Unlike most of the methods discussed above, oxidimetric determination with Brcl is simple, precise and accurate.

EFFECT OF REACTION TIME:- Keeping the amount of cysteine , concentration of BrCl reagent and acetic acid as constant, the reaction time was varried from 1-30 minutes. Aliquots containing 1-5 mg. of cysteine were taken in 100mg Erlenmeyer flask and 1 ml of 0.05 N BrCl reagent and The reaction was carried out in Ice bath for 1, 5, 10, 15, 20, 25 minutes. After the prescribed reaction time contents were cooled to room temperature and the unconsumed BrCl reagent was Estimated iodometrically. It was observed that the recovery of the sample becomes constant within there action of 15 minutes . Thus for general procedure are action time of 15 mintues was recommended. A lesser reaction time gives lower results while prolonged reaction time has no effect on the recovery of the sample. Keeping amount of cysteine and reaction time as constant, the effect of varying concentration of BrCl reagent was studied. The concentration was varied from 0.01 N to 0.06 N and the recovery of the sample was calculated. It was found that the best recovery of the sample was obtained by using 0.05 N concentration of the reagent. (Table-2)

EFFECT OF VOLUME OF BrCl

The effect of volume of 0.05 BrCl reagent on there covery of sample was also studied. It was noted that 1ml. of 0.05 N BrCl reagent gives best recovery. Thus for a general procedure 1ml. of 0.05 N BrCl reagent was recommended. (Table-3)

EFFECT OF TEMPERATURE : While studying the effect of reaction temperature on the recovery of cysteine was found that the reaction gives accurate on ice bath give best result at 5^0 the temperature was maintained thermostatically. (Table-4)

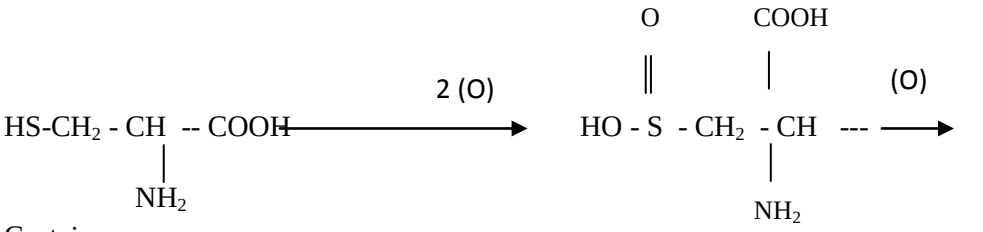
After studying various variables following reaction conditions were developed.

Aliquots containing 1-5 mg. of the sample were taken in a 100 ml. Erlenmeyer flask followed by the addition of 1 ml. of 0.05 N BrCl reagent . The reaction was carried out in ice bath. After the prescribed reaction time, contents unconsumed BrCl reagent was determined iodimetrically with Hypo solution using starch as indicator. A blank experiment was also run under idential condition using all the reagent except the sample. The volume BrCl reagent consumed per mole of sample were noted and the recovery of the sample was calculated.

ESTIMATION OF THIO AMINO ACIDS WITH 0.05 N. BrCl REAGENT BY MICRO METHODS :

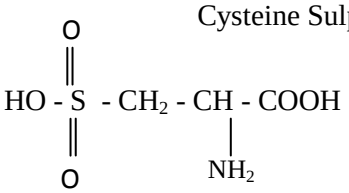
With the recommended procedure Estimation of cysteine or methionine has successfully been achieved (table 6-9)

RESULTS AND DISCUSSION :- The oxidation of thioamine acids had widely been studied by other workers and then course of the reaction has been suggested. In many cases, it is observed that the sulphur function gets oxidised to sulphonic acid group. As reported earlier, the oxidation of cysteine with N-bromosuccinimide, performic acid[26] and ammoniumhexanitratocerate (IV) proceeds through following mechanisms

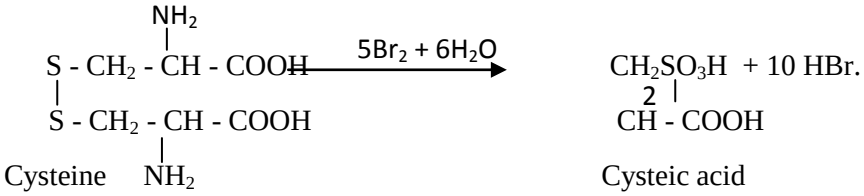


Cysteine

Cysteine Sulphonic acid



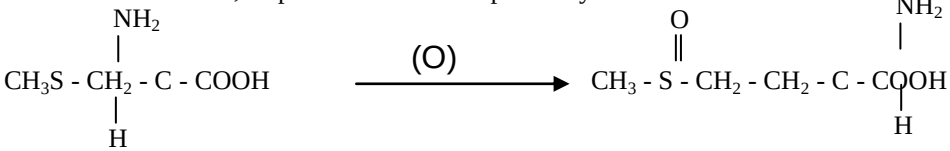
The oxidation of cystine which contains disulphide function, with the use of N-bromosuceinimide and bromine[29] has also been carried out. It is assumed that it is also oxidised to cystiec acid which is supported by other workers. [26-27]



Cysteine

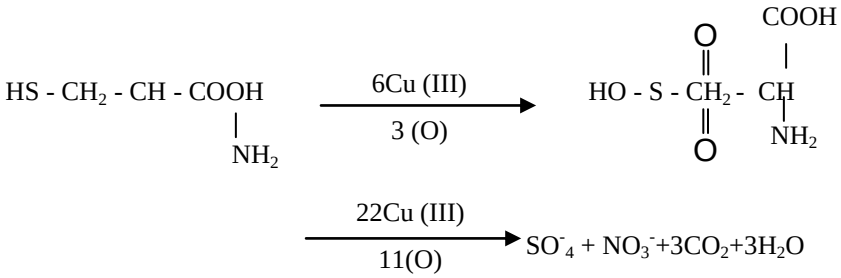
Cysteic acid

In case of methionine, sulphoxide has been reported by several workers[24,30,31]

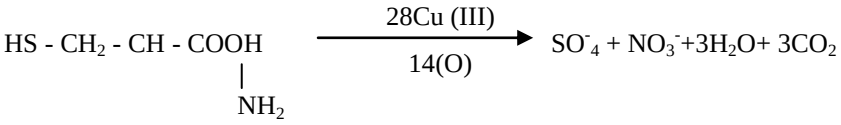


Methionine sulphoxide

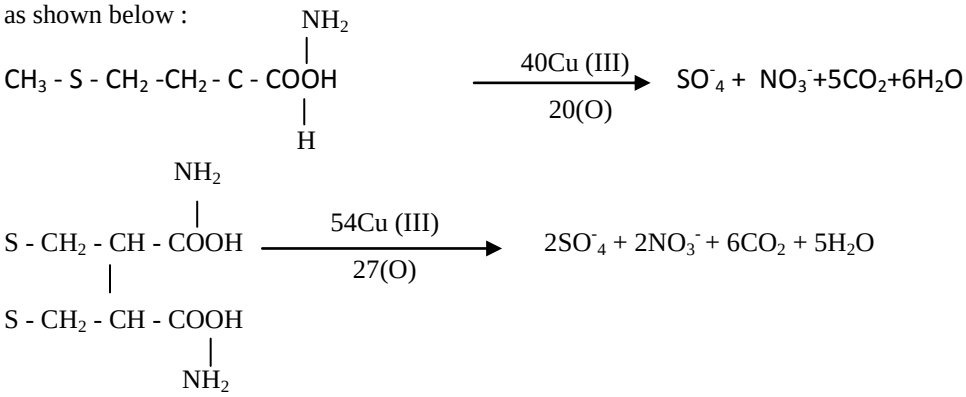
Based on the reaction shown above, by the knowledge of stoichiometry in each case and confirming the presence of SO_4 ion in the reaction mixture. Complete oxidation of thioaminoacids may be suggested with Cu (III) reagent.[35]



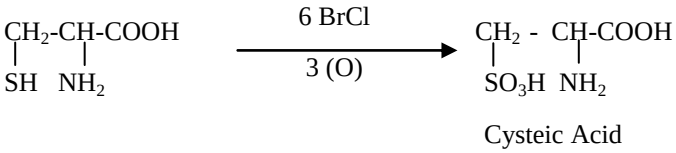
Overall reaction may be shown :



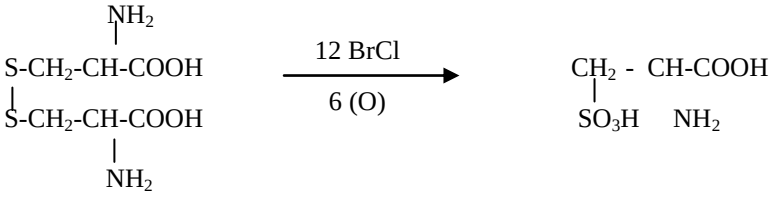
Similarly the consumption of 40 and 54 moles of Cu (III) in case of methionine and cystine respectively may be suggested as shown below :



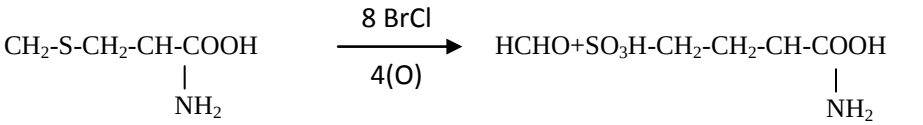
Considering the oxidation reaction of thioamino acids and the number of equivalent of BrCl consumed for a particular sample the following course of reaction may be suggested for the oxidation of cysteine, cystine and methionine.



Cysteic Acid



Cyteine Cysteic Acid



Methionine Homocysteic acid

The above mechanism find supports from previous mechanism[27]

PREPARATION OF SAMPLE SOLUTION

The samples were weighed accurately and dissolved in minimum amount of acetic acid and made up to the volume with cold distilled water in volumetric flask to give a concentration of 1 Mg./ml. All the samples were of Baker analysed reagent and purity was tested by their melting point determined.

GENERAL PROCEDURE

Aliquots containing 1-9 mg. of the sample was placed in a 100 ml. iodine flask followed by addition of 5 ml. of glacial acetic acid, 10 ml. 0.05 N solution of bromine monochloride was added to it and the contents were shaken thoroughly. The flask was stoppered, placed in ice bath containing ice salt mixture and allowed to react for 5 minutes with occasional shaking. the cystein, cystine , methionine reaction was allowed to proceed for 15 minutes. After the reaction was over the stopper was washed with 5 ml. of distilled water and 10 ml. of potassium iodide (15% was added to it. Contents were shaken thoroughly and kept for a minute. The liberated iodine was titrated with standardised 0.02 N sodium thiosulphate solution using starch as indicator. A blank experiment was also performed under identical conditions using all the re-agent except the sample.

CALCULATION

$$\text{mg. of sample} = \frac{(B-A) \times M \times N}{2 \times n}$$

A = ml. sodium thiosulphate for sample.
B = ml. sodium thiosulphate for blank.
N = Normality of sodium thiosulphate solution
M = Molecular weight of the sample
n = Moles of brominemonochloride for the sample.

Table-1

Effect of reaction time on the recovery of Cysteine with BrCl reagent.

Aliquots taken (ml)	Amount present (mg)	Reaction time (min)	Amount recovered (mg)	Error %
2	2.00000	1	1.0638	-1.81
2	2.00000	5	1.0830	-0.85
2	2.00000	10	1.0930	-0.35
2	2.00000	15	2.0010	+0.05
2	2.00000	20	2.0088	+0.44
2	2.00000	25	2.0110	+0.55

TABLE – 2 Effect of concentration of BrCl reagent on the recovery of Cysteine

Aliquots taken (ml)	Amount present (mg)	Strength of Brcl (N)	Amount recovered (mg)	Error %
2	2.00000	0.01	1.0880	-0.60
2	2.00000	0.03	1.0920	-0.018
2	2.00000	0.05	2.0010	+0.05
2	2.00000	0.06	2.0135	+0.67

TABLE – 3 Effect of Volume of 0.05 N BrCl reagent on the recovery of Cysteine

Aliquots taken (ml)	Amount present (mg)	Volume of Brcl (ml)	Amount recovered (mg)	Error %
2	2.00000	0.5	1.0880	-0.60
2	2.00000	1.0	2.0018	+0.09
2	2.00000	1.5	2.0120	+0.60
2	2.00000	2.0	2.0135	+0.63
2	2.00000	3.0	2.0170	+0.85

In each case three determination were done.

TABLE-4 Effect of reaction temperature on the recovery of Cysteine

Aliquots taken (ml)	Amount present (mg)	Temperature (^{O}C)	Amount recovered (mg)	Error %
2	2.00000	15	1.0900	-0.50
2	2.00000	10	1.0930	-0.35
2	2.00000	5	2.0110	+0.53
2	2.00000	4	2.0180	+0.90
2	2.00000	2	2.0196	+0.98

In each case three determination were done.

Table-5 Stoichimetric ratios of some thioamino acids with BrCl reagent

Sample		Observed molar ratios of BrCl per mole of compounds		
1.	Cysteine	5.9990	6.0080	6.0050
2.	Cystine	12.0040	11.9900	12.0100
3.	Methionine	8.0070	8.0030	7.9990

In each case three determination were done.

TABLE-6 Microdetermination of cysteine with 0.05 N BrCl reagent

Aliquots taken (ml)	Amount present (mg)	Reaction time (min)	Molecularity	Amount recovered (mg)	Error %
1	1.0000	15	6	0.9958	-0.42
				0.9969	-0.31
				0.9990	-0.10
2	3.0000	15	6	3.0075	+0.25
				2.9910	-0.30
				2.9910	-0.30
5	5.0000	15	6	5.0200	+0.40
				5.0175	+0.35
				5.0155	+0.31
7	7.0000	15	6	7.0406	+0.58

				7.0420	+0.60
				7.0434	+0.62
9	9.0000	15	6	8.9865	-0.15
				8.9712	-0.32
				8.9775	-0.25

In each case three determination were done.

TABLE- 7 Estimation of cysteine with 0.05 N BrCl reagent

Aliquots taken (ml)	Amount present (mg)	Reaction time (min)	Molecularity	Amount recovered (mg)	Error %
1	1.0000	15	12	1.0051	+0.51
				1.0065	+0.65
				1.0047	+0.47
3	3.0000	15	12	2.9922	-0.26
				3.0084	+0.28
				3.0126	+0.42
5	5.0000	15	12	5.0155	+0.31
				5.0180	+0.36
				4.9930	-0.14
7	7.0000	15	12	6.9832	-0.24
				6.9825	-0.24
				6.9755	-0.35
9	9.0000	15	12	9.0261	+0.29
				9.0342	+0.38
				9.0216	+0.24

* In each case three determination were done.

TABLE- 8 Estimation of Methionine with 0.05 N BrCl reagent

Aliquots taken (ml)	Amount present (mg)	Reaction time (min)	Molecularity	Amount recovered (mg)	Error %
1	1.0000	15	8	1.0025	+0.25
				1.0023	+0.23
				0.9983	-0.17
3	3.0000	15	8	2.9925	-0.25
				3.0096	+0.23
				3.0066	+0.22
5	5.0000	15	8	4.9860	-0.28
				5.0070	+0.14
				4.9825	-0.35
7	7.0000	15	8	7.0189	+0.27
				7.0280	+0.40
				7.0112	+0.16
9	9.0000	15	8	8.9712	-0.32
				8.9766	-0.26
				9.0117	+0.13

In each case three determination were done.

TABLE- 9 Microdetermination of some Thioaminoacids with the recommended procedure with 0.05 N BrCl reagent.

Sample	Amount taken (mg)	Reaction time (min)	Stoichio-metry	Amount recovered (mg)	% of amount obtained by calc.	Error %
1. Cysteine	1.0000	15	6	0.9958	99.83	-0.42
				0.9969		-0.31
				0.9990		-0.10
	3.0000			3.0075		+0.25
				2.9910		-0.30
				2.9910		-0.30
	5.0000			5.0200		+0.40
				5.0175		+0.35
				5.0155		+0.31
2. Cystine	1.0000	15	12	1.0025	100.01	+0.25
				1.0023		+0.23
				0.9983		-0.17
	3.0000			2.9925		-0.25
				3.0096		+0.23
				3.0066		+0.22
	5.0000			4.9860		-0.28
				5.0070		+0.14
				4.9825		-0.35
3. Methionine	1.0000	15	8	1.0051	100.28	+0.51
				1.0061		+0.65
				1.0047		+0.47
	3.0000			2.9922		+0.26
				3.0084		+0.28
				3.0126		+0.42
	5.0000			5.0155		+0.31
				5.0180		+0.36
				4.9930		-0.14

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